Biochem. J. (1985) **229**, 101–108
Printed in Great Britain

Experimentally induced defects of mitochondrial metabolism in rat skeletal muscle

Biological effects of the mitochondrial uncoupling agent 2,4-dinitrophenol

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(*Received 7 December 1984/28 February 1985; accepted 11 March 1985*)

Infusion of dinitrophenol intra-arterially into rat hind limb caused an irreversible failure of isometric twitch tension and the induction of a severe progressive contracture. Metabolite analysis of muscle in which the twitch response had grossly fatigued revealed low levels of ATP and phosphocreatine together with lactate accumulation. Studies using ^{31}P-n.m.r. confirmed the decrease in ATP and creatine phosphate concentrations and indicated a fall in intracellular pH. It is concluded that dinitrophenol-induced myopathy does not represent a good model for the human mitochondrial myopathic condition as has been previously suggested.

The relationship between ATP production, whether in the cytosol by glycolysis, or intramitochondrially by oxidative phosphorylation, and the energy demands of muscle function continues to be a matter of considerable interest. In particular this relationship is the focus of attention in a group of human diseases referred to as the 'mitochondrial myopathies' (DiMauro, 1979; Morgan-Hughes, 1983) in which muscle function is impaired possibly because of a defective provision of ATP for muscle contraction. Whilst there are a number of different defects, two relate directly to the process of oxidative phosphorylation: (*a*) a defect of a component of the respiratory chain itself, and (*b*) a defect in the coupling of the respiratory chain to the phosphorylation system (Luft's Syndrome; Luft *et al.*, 1962). Dinitrophenol is a classical mitochondrial uncoupling agent whose effects on skeletal muscle metabolism have been studied for many years. Early investigations *in vitro* showed that this agent caused contracture and the depletion of adenine nucleotides and creatine phosphate in frog and rat diaphragm muscle (Cori & Cori, 1936; Weeks & Chenowett, 1952; Barnes *et al.*, 1955). More recently, studies *in vivo* have indicated similar results together with altered morphological features of the muscle mitochondria (Melmed *et al.*, 1975; Saghal *et al.*, 1979; Walter *et al.*, 1981). Further, it has been suggested that intra-arterial infusion of dinitrophenol may represent a useful model for the mitochondrial myopathies, although classical uncoupling of oxidative phosphorylation has not been encountered clinically.

A detailed study of both the physiology and biochemistry of the effects *in vivo* of dinitrophenol on rat skeletal muscle function is reported here. The main finding was that dinitrophenol caused a major decline in muscle twitch response followed by a variable degree of contracture. This is discussed with respect to changes in the muscle concentrations of the nucleotides, creatine phosphate and lactate.

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Materials and methods

Materials

2,4-Dinitrophenol (Sigma Chemical Co.) was dissolved in 1M-NaOH by gentle warming to a final concentration of 10mg/ml. The solution was adjusted to a final pH of 7.4 with 10M-HCl. The ion-pairing agent, 11-aminoundecanoic acid used for h.p.l.c. was also obtained from Sigma.

Muscle physiology

Adult Wistar rats (180–340g) were anaesthetized with urethane [intraperitoneal; 25% (w/v) in saline, 5ml/kg body wt.]. A polyethylene cannula was positioned distally to the aortic bifurcation through a left femoral arteriotomy. The right gastrocnemius muscle was exposed distally, separated from the soleus and plantaris muscle, and attached to a strain gauge (braided steel tie < 1 mm length, Statham UC3 gauge, L4S accessory diaphragm, undamped oscillation frequency 1200Hz, linear response over 5kg range). The right hand limb was immobilized in a rigid animal frame with stainless steel pins 2cm apart fixing the femur. Fine silver electrodes were placed on the right sciatic nerve and around the body of the gastrocnemius for nerve and muscle stimulation respectively. Supramaximal shocks (50μs, 50V) were used for nerve stimulation and 200μs, 300V shocks for muscle stimulation. Action potentials were recorded through a Teflon-coated silver wire electrode with the distal 5mm bared and inserted into the end plate zone with the indifferent electrode in the right foot. The muscle was kept moist throughout recording with physiological (140mM-NaCl/4mM-KCl/2.4mM-$CaCl_2$) saline warmed to 37°C. Muscle surface temperatures varied between 28 and 32°C.

Muscle biochemistry

Metabolite measurement. The gastrocnemius muscle was rapidly freeze-clamped between two brass plates at 77K, quickly transferred to liquid N_2 and stored at −70°C prior to analysis. The nucleotides were extracted in 10vol. of 10% (w/v) trichloroacetic acid in 20% (w/v) methanol (see Lush *et al.*, 1979). After five washes with 5vol. of water-saturated diethyl ether the extract was adjusted to pH7.0 by the addition of solid Tris. The nucleotides were measured by using a modification of the h.p.l.c. method of Knox & Jurrand (1981). The mobile phase consisted of 95mM-KH_2PO_4/2mM-11-aminoundecanoic acid/12% (v/v) methanol, pH5.66 (adjusted with 150mM-Na_2HPO_4) and the column was an ODS (5μm; theoretical plates $\geqslant 60000 m^{-1}$) size 10cm × 0.45cm. The eluted nucleotides were detected at 254nm and quantified with respect to standard nucleotide chromatographs on an Hewlett Packard (HP 3308A) integrator/plotter. The order of elution was inosine, NAD, IMP, AMP, ADP and ATP, with typical total analysis times of 25min. Samples for lactate and phosphocreatine estimations were extracted in 10vol. of 10% (w/v) $HClO_4$ and assayed by standard spectrophotometric assays (Bergmeyer, 1974).

Calculation of metabolite concentrations. The ATP values obtained by h.p.l.c. were in good agreement with enzymically determined values (results not shown) and were assumed to be entirely n.m.r.-visible (see Dawson *et al.*, 1980; Meyer *et al.*, 1982). The conditions under which muscles were extracted for lactate and phosphocreatine estimations were not optimal for phosphocreatine. From a series of experiments in which muscle extracts were spiked with known concentrations of phosphocreatine (5–20mM) it was shown that an average 43 ± 3% (mean ± S.D., $n = 9$) of the phosphocreatine was hydrolysed. Subsequent experiments have shown that this can be prevented entirely by using 5vol. of 5% (w/v) $HClO_4$ in place of the 10vol. of 10% (w/v) $HClO_4$. In both conditions, however, a loss of 20–25% of phosphocreatine due to freeze-clamping cannot be avoided. Phosphocreatine levels were, therefore, corrected for loss due to freeze-clamping and subsequent hydrolysis in the extraction procedure. By collecting fully relaxed n.m.r. spectra at rest and by using the mean ATP concentration (Table 1), the phosphocreatine concentration *in vivo* (27.7 ± 2.5μmol/g wet wt.; mean ± S.D., $n = 5$) was calculated from their relative peak integrals (see Shoubridge & Radda, 1984). This involved a correction factor of 3.12 by which the spectrophotometrically determined levels of phosphocreatinine were multiplied.

Nuclear magnetic resonance

All experiments were done at 73.84MHz in a vertical, wide-bore 4.3T magnet. A three-turn surface coil, 14mm outside diameter, was placed on the gastrocnemius muscle and served as transmitter and receiver. The characteristics of the surface coil are such that the intensity of the signal is negligible a radius away from the plane of the coil (Ackerman *et al.*, 1980). In terms of the experiments described here, this means that mainly gastrocnemius muscle is being observed, with a small contribution from the plantaris and a negligible contribution from the soleus muscle.

Spectra were collected as 2K data points using the following parameters: sweep width ±2000Hz, pulse width 20μs, recycle time 2s and were zero-filled. All spectra were referenced to the proton resonance of tissue water which was set at zero after adjusting the magnetic field homogeneity. In this system phosphocreatine appears at 5.2p.p.m. downfield from the origin in the ^{31}P spectrum. Spectra were quantified by using peak intensities and pH was determined from the chemical shift of inorganic phosphate (Moon & Richards, 1973; Gadian *et al.*, 1979). Recordings were made at rest and with sciatic nerve stimulation in different experiments. Twitch tension was recorded in most experiments, although not under strict isometric conditions.

Results

Muscle physiology

Three stimulation protocols were investigated: (*a*) 0.033 Hz (i.e. 2 cycles/min), (*b*) 5 Hz, and (*c*) tetanic trains (100 Hz for 2 s/min).

Stimulation at 0.033 Hz. The gastrocnemius muscle was stimulated once by direct muscle activation and once via the sciatic nerve every 1 min. The dinitrophenol was infused over 10 min (total dose 16 mg/kg body wt.). The isometric twitch tension began to fall within a few minutes of dinitrophenol infusion [mean time of onset 5.3 ± 2.3 min (mean $\pm$ S.D., $n = 23$)] followed by an electrically silent rise in the resting tension or contracture [mean time of contracture onset 10.6 ± 2.7 min (mean $\pm$ S.D., $n = 23$)]. The pattern of twitch failure was identical with either muscle or nerve stimulation (see Fig. 1). A major fall in twitch tension (mean value 34%, range 20–60% of the initial response) always preceded contracture development. The compound muscle action potential also failed following dinitrophenol infusion (Fig. 1).

Stimulation at 5 Hz. In 12 experiments the gastrocnemius muscle was stimulated at 5 Hz (seven via the nerve and five via direct muscle stimulation), and the twitch response stabilized. This 'steady state' twitch tension was stable in control experiments for several hours (see also Edstrom & Kugelberg, 1968; Kugelberg & Edstrom, 1968). Once the steady state twitch response was reached, boluses of dinitrophenol (4 mg/kg body wt.) were infused (Fig. 2). With each infusion of dinitrophenol there was a stepwise decline in muscle action potential amplitude and twitch tension. This pattern was reproduced following each infusion and after three or four infusions both parameters failed completely. If the infusions of dinitrophenol (total dose 16 mg/kg body wt.) were initiated at the onset of the 5 Hz stimulation trains, the normal steady state plateau response was not observed. The subsequent fall in the twitch response was not a stepwise decline (as above) but a sharp fall within 5 min of stimulation. The development of a progressive contracture was also a feature.

Tetanic stimulation. Brief tetanic pulses (100 Hz for 2 s/min) by alternating nerve and direct muscle stimulation elicited stable tension plateaus in control experiments for up to 30 min. The infusion of dinitrophenol was given in one dose at 1 min (12 mg/kg body wt.). This caused a rapid severe force failure by both stimulus routes. However the peak tetanic tension was greater with direct muscle activation and the degree of tension fatigue was less than seen with nerve stimulation (results not shown). This suggested that failure of neuromuscular transmission made an early contribution to force failure in this situation.

Metabolite content of gastrocnemius muscle infused with dinitrophenol stimulated at 0.033 Hz

The stimulated gastrocnemius muscles were freeze-clamped at three time points. The metabo-

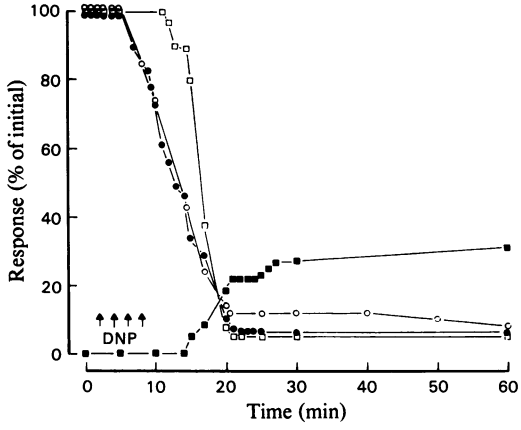

Fig. 1. *Dinitrophenol infusion during stimulation at 0.033 Hz*
A typical experiment in which dinitrophenol (DNP) was infused over 10 min (final dose 16 mg/kg body wt.) is shown. Twitch tension was recorded at the times indicated with stimulation via nerve (●) or by direct muscle activation (○). Contracture development (■) and compound muscle action potential (□) is also plotted. Note that the pattern of twitch failure is the same for both mechanisms of muscle stimulation.

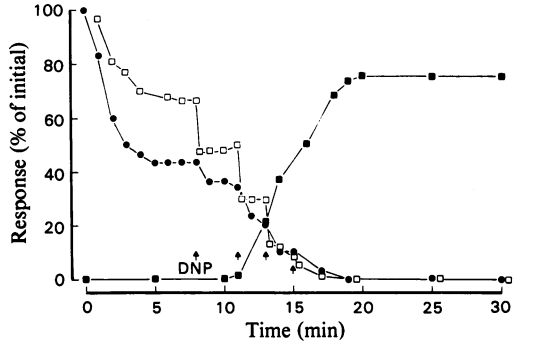

Fig. 2. *Dinitrophenol infusion during stimulation at 5 Hz*
A typical experiment in which the gastrocnemius muscle was stimulated via the nerve at 5 Hz. Once the steady state twitch tension was reached boluses (4 mg/kg body wt.) of dinitrophenol (DNP) were infused at the times indicated by the arrows. Note the step-like decline in the twitch tension (●) and in the action potential (□). Contracture (■) only evolved following a major fall in the twitch tension.

lite content of the muscles displayed a degree of variability with respect to time (Table 1), but an overall general trend could be established. There was a significant depletion in high energy phosphate compounds (ATP, phosphocreatine) and a marked increase in muscle lactate concentrations. The levels of ATP, the ATP/ADP ratio and the phosphocreatine concentrations were significantly lower than control values at all three time points. However comparison within the experimental groups of these three values revealed only one significant difference, which was between the phosphocreatine levels at 15 and 30 min (Table 1). It would appear that the major metabolic changes have occurred by 15 min and that the system has deteriorated irreversibly. However, in some instances where the twitch response was well maintained (> 50% of the initial value), the levels of ATP and phosphocreatine were higher than other members of their respective time groups. To rationalize the variability in both the physiological and biochemical parameters these parameters were compared by means of least squares linear regression analysis. The reduced levels of ATP, ATP/ADP ratio and phosphocreatine correlated with twitch response [correlation coefficients (r) of 0.85, 0.94 and 0.85 respectively] (see Fig. 3*a*) but less well with contracture evolution (r values of 0.70, 0.56 and 0.49 respectively). However the increased levels of inosine and IMP correlated with contracture ($r = 0.83$ and 0.71 respectively; see Fig. 3*b* for the situation for inosine).

Metabolite content of gastrocnemius muscle infused with dinitrophenol stimulated at 5 Hz

In this set of experiments both experimental (i.e. dinitrophenol-infused) and control muscles were subjected to a 5 Hz stimulation for 20–30 min respectively (Table 2). This contrasts with the previous set of data (Table 1) where the muscles were essentially at rest during the whole period of the dinitrophenol infusion. When the control stimulated muscle metabolite profile (Table 2) is compared with the experimental muscle profile, phosphocreatine and the adenine nucleotide pool has decreased significantly whereas the lactate and IMP concentrations have increased markedly in the experimental muscle.

^{31}P-n.m.r. studies

The infusion of dinitrophenol was studied without muscle stimulation by using ^{31}P-n.m.r. spectroscopy. The infusion of dinitrophenol (16 mg/kg body wt.) caused a steady decline in the phosphocreatine signal and the pH fell (Fig. 4), followed by a decrease in the ATP signals. These results indicated that infusion of dinitrophenol caused depletion in high energy phosphate compounds, presumably by increasing ATP hydrolysis [since this agent stimulates endogenous ATPases:

Table 1. *Metabolite levels in rat gastrocnemius muscle infused with dinitrophenol and stimulated at 0.033 Hz*
The muscle was infused intra-arterially with dinitrophenol (16 mg/kg body wt. in four equal size boluses at 2 min intervals), stimulated at 0.033 Hz and freeze-clamped at the times shown. Metabolite assays were carried out by h.p.l.c. and as indicated in the Materials and methods section. Student's t-test was used to compare the four groups: control versus all experimental groups, $^{a}P < 0.005$, $^{b}P < 0.02$, $^{c}P < 0.01$; 15 min group versus 30 min group, $^{d}P < 0.02$; $^{e}P < 0.01$; 20 min group versus 30 min group, $^{f}P < 0.02$; ND, not determined. Results are expressed as μmol/g wet wt. and are means ± S.D. for at least three separate experiments.

		Level (μmol/g wet wt.)			
Metabolite	Infusion time (min) . . .	0	15	20	30
Inosine		0.22 ± 0.04	0.12 ± 0.06	ND	0.40 ± 0.13
NAD		0.49 ± 0.02	0.36 ± 0.16	0.36 ± 0.04^{b}	0.39 ± 0.04^{c}
IMP		0.52 ± 0.08	0.86 ± 0.31	2.61 ± 1.69	4.00 ± 2.4
AMP		0.004 ± 0.001	0.05 ± 0.02^{b}	0.19 ± 0.12	0.14 ± 0.03
ADP		0.78 ± 0.08	0.92 ± 0.39	1.16 ± 0.29	1.00 ± 0.52
ATP		7.60 ± 0.04	3.62 ± 1.73^{b}	2.08 ± 0.55^{c}	2.06 ± 1.9^{b}
ATP/ADP		9.8 ± 1.2	3.9 ± 0.4	2.2 ± 1.6^{b}	1.8 ± 0.9^{bf}
Total adenine nucleotides		8.39 ± 0.13	4.59 ± 2.1^{b}	3.43 ± 0.81^{c}	3.20 ± 2.39
Lactate		1.79 ± 0.64	10.15 ± 3.0^{b}	11.6 ± 7.5	14.4 ± 4.9^{b}
Phosphocreatine		27.74 ± 2.52	4.82 ± 1.61^{c}	4.24 ± 1.27^{bc}	0.09 ± 0.15^{bd}
Adenylate energy charge		0.95 ± 0.01	0.89 ± 0.01	0.78 ± 0.09^{a}	0.77 ± 0.1^{a}
Twitch tension (%)		100	49 ± 9	19 ± 28^{b}	19 ± 29^{b}
Contracture (%)		0	13 ± 10	22 ± 10^{a}	35 ± 17

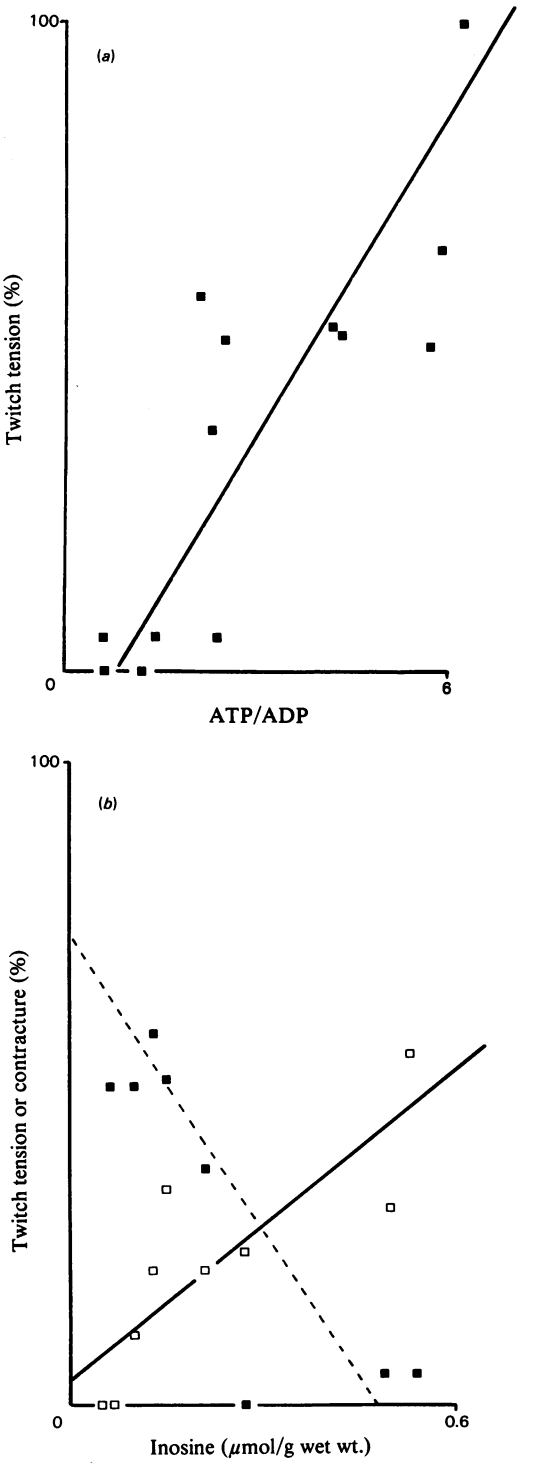

Fig. 3. *Rat gastrocnemius muscle infused at rest with dinitrophenol*

The muscle was infused intra-arterially with dinitrophenol (16mg/kg body wt. in four equal size boluses at 2min intervals), stimulated at 0.033Hz and freeze-clamped at the specified times shown in Table 1. Metabolite assay was carried out by h.p.l.c. as indicated in the Materials and methods section. (*a*) ATP/ADP ratio versus twitch tension (%). Regression analysis: $y = 17.4x - 14.9$: $r = 0.936$. (*b*) Inosine concentration (μmol/g wet wt.) versus twitch tension (%) (■) or contracture (%) (□). Regression analysis: twitch tension, $y = 147x + 72.2$, $r = 0.824$; contracture, $y = 799x + 4.3$, $r = 0.832$.

see Levy *et al.* (1963)]. In a further group the dinitrophenol was infused (12mg/kg body wt.) once the twitch response had stabilized at its steady state level (74% of the initial response) after 1 Hz stimulation (Fig. 5). The twitch response and the phosphocreatine level fell rapidly and again a progressive contracture was noted. No recovery in either twitch tension or phosphocreatine was observed following cessation of stimulation.

In summary, infusion of dinitrophenol into rat hind limb caused a rapid muscle fatigue response coupled to depletion of ATP and phosphocreatine. These events were followed by a progressive contracture.

Discussion

The exposure of rat skeletal muscle *in vivo* to the uncoupling agent, 2,4-dinitrophenol induced an

Table 2. *Metabolite levels in rat gastrocnemius muscle infused with dinitrophenol and stimulated at 5 Hz*

Rat gastrocnemius muscle was stimulated at 5Hz and freeze clamped for metabolite analysis. The control groups were stimulated for 30min and the experimental group for 20min. Dinitrophenol at a dose of 16mg/kg body wt. was infused from the beginning of the muscle stimulation in the experimental group. Values are means ± S.D. for four experiments. Significance by Student's *t*-test: $^{a}P < 0.001$, $^{b}P < 0.002$, $^{c}P < 0.005$, $^{d}P < 0.01$.

	Level (μmol/g wet wt.)	
Metabolite	Control	Experimental
Inosine	0.28 ± 0.07	0.28 ± 0.04
NAD	0.50 ± 0.03	0.35 ± 0.04
IMP	1.71 ± 0.41	5.17 ± 0.97[c]
AMP	0.06 ± 0.02	0.15 ± 0.07
ADP	0.92 ± 0.10	0.63 ± 0.11[d]
ATP	3.59 ± 0.19	0.68 ± 0.31[a]
ATP/ADP	3.91 ± 0.6	1.2 ± 0.3[a]
Total adenine nucleotides	4.56 ± 0.13	1.42 ± 0.42[a]
Lactate	7.31 ± 1.74	16.41 ± 0.41[a]
Phosphocreatine	7.15 ± 0.60	0.73 ± 0.70[a]
Adenylate energy charge	0.90 ± 0.01	0.68 ± 0.09[d]
Twitch tension (%)	56 ± 16	1 ± 1[b]
Contracture (%)	0	50 ± 59

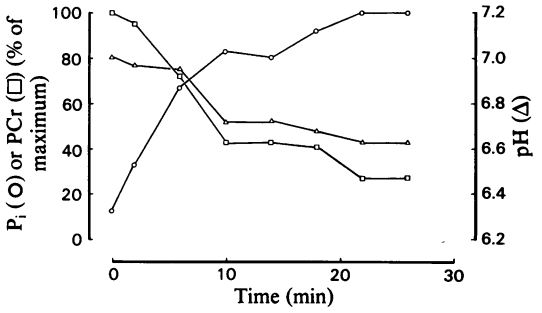

Fig. 4. *^{31}P-n.m.r. observations on rat gastrocnemius muscle infused at rest with dinitrophenol*

A typical experiment is shown. The animals were cannulated and mounted in the n.m.r. spectrometer (see the Materials and methods section). Two spectra were collected at rest and dinitrophenol was then infused (16mg/kg body wt. over 8min) and further spectra were collected. The metabolite profiles are calculated as a percentage of the maximum value obtained in the particular experiment (see Table 1 for control mean values); the variation of the n.m.r. measurements was ±10%. □, Phosphocreatine; ○, P_i; △, pH.

irreversible failure of twitch tension and muscle action potential with the onset of severe contracture. At low stimulation frequencies the muscle action potential is relatively well maintained at a time when the mechanical response to direct or nerve stimulation is rapidly declining (Fig. 1). With higher stimulation frequencies there is a parallel decline in both parameters (Fig. 2). With tetanic stimulations, force fatigue developed more rapidly with nerve than with direct muscle stimulation, indicating that neuromuscular fatigue is unmasked at higher activation frequencies.

During steady state stimulation (5Hz) the supply of energy is mainly oxidative (Edstrom & Kugelberg, 1968; Kugelberg & Edstrom, 1968). The rapid decline in twitch tension following each bolus of dinitrophenol reflects the dependence of force maintenance on oxidative phosphorylation. The steplike fall in twitch tension seen with repeated dinitrophenol bolus injections during a 5Hz pattern (Fig. 2) suggested that there was a variable range of muscle fibre impairment following the infusion of each bolus. Subsarcolemmal mitochondria may be more susceptible to the action of uncoupler by virtue of their location. Therefore it was probable that each bolus of dinitrophenol produced a wide spectrum of mitochondrial impairment in any one fibre.

Dinitrophenol-induced contracture of skeletal muscle *in vitro* has been recognized for many years (Cori & Cori, 1936; Weeks & Chenowett, 1952; Barnes *et al.*, 1955). A similar evolution of contracture was demonstrated *in vivo* in this study. The clear differentiation between the onset of twitch failure and contracture in all experiments, with the former falling by as much as 60% of initial tension before contracture began, suggested that in any one fibre (or contractile subunit) the processes of twitch failure and contracture were not closely related. Failure of the twitch response must be followed by a brief period of relaxation before contracture evolves. The final contracture tension which evolved in different experiments was quite variable, suggesting that either a variable number of fibres entered contracture or that there was a considerable range in the number of rigor complexes formed in each fibre (see Weber & Murray, 1973).

Analysis of dinitrophenol-poisoned muscle by ^{31}P-n.m.r. and conventional biochemical techniques showed severe depletion of phosphocreatine and ATP with accumulation of lactate. The rapid phosphocreatine hydrolysis in resting muscle demonstrated by ^{31}P-n.m.r. (Fig. 4) suggested that dinitrophenol accelerated ATP hydrolysis as well as uncoupled oxidative phosphorylation. This was in keeping with observations in cardiac muscle *in vitro* where ATP hydrolysis was much more rapid after exposure to dinitrophenol than with either ischaemia or cyanide poisoning (McDonald & Macleod, 1972). Dinitrophenol is also known to interact directly with myosin ATPase as well as mitochondrial ATPase (Levy *et al.*, 1963) and this may lead to ATP hydrolysis which is not associated with the normal contraction-induced hydrolysis. Hydrolysis of myosin–ADP–P_i complexes (see Weber & Murray, 1973) may accelerate rigor complex formation. Furthermore, ATP depletion correlated well with contraction failure, in keeping with earlier observations in frog muscle (Abood *et al.*, 1961) and in rat muscle *in vitro* (Barnes *et al.*, 1955). In normal muscle fatigue, by contrast, observations both in human and in frog muscle indicate that contraction stops as glycolysis and phosphocreatine levels become exhausted at a time when ATP concentrations are relatively preserved (Wilkie, 1981). Acceleration of ATP hydrolysis by dinitrophenol could account for this difference and would lead inexorably to severe energy depletion and contracture. Although ATP depletion and contracture evolution correlated very closely in individual experiments, the interexperiment variation in contracture extent at similar ATP levels suggests that other factors are important in its evolution. Calcium accumulation secondary to the impairment of mitochondrial (Carafoli & Rossi, 1971) and energy-dependent sarcoplasmic reticulum calcium reuptake probably has a synergistic effect.

Several key differences emerged between the physiological findings in acute dinitrophenol myo-

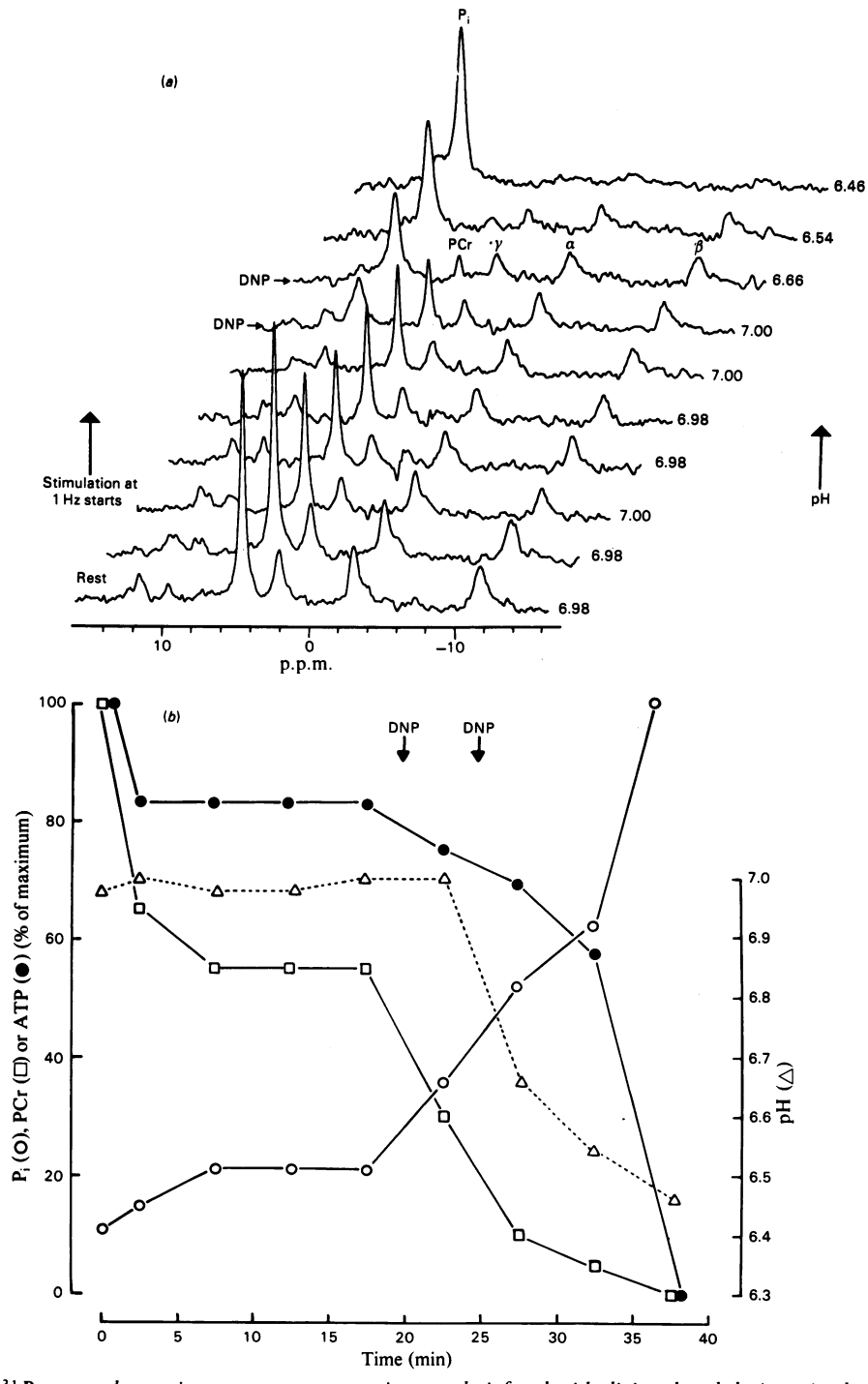

Fig. 5. *^{31}P-n.m.r. observations on rat gastrocnemius muscle infused with dinitrophenol during stimulation at 1 Hz*
(*a*) A typical experiment is shown. N.m.r. spectra were collected over 4.8 min periods. Dinitrophenol (DNP) (12 mg/kg body wt.) was infused at the times indicated. The pH is shown at the right hand side of each spectra. α, β, and γ represent the α-, β-, and γ-phosphate groups of ATP, PCr represents phosphocreatine and P_i inorganic phosphate. (*b*) The metabolite profiles from (*a*) have been calculated and plotted in a similar fashion to Fig. 4, i.e. as a percentage of the maximum value obtained in the particular experiment. In the absence of added dinitrophenol (see arrows) the percentage of the initial resting value of phosphocreatine and pH would remain constant over the time course of the experiment. □, Phosphocreatine; ○, P_i; △, pH; ●, ATP.

pathy and those reported in patients with mitochondrial myopathies. The massive irreversible contracture seen in this study and mentioned by Melmed *et al.* (1975) and Saghal *et al.* (1979) is not encountered clinically in patients. A failure of excitation coupling or contractility has been demonstrated in fatigue in mitochondrial myopathic patients (De Jesus, 1974; Morgan-Hughes *et al.*, 1977, 1984; Wiles *et al.*, 1981) but in dinitrophenol-induced myopathy the inexcitability of the sarcolemma was closely associated with force failure. These differences reflect the greater severity of the biochemical dysfunction observed with dinitrophenol exposure. Thus, although the acute dinitrophenol myopathy is of considerable interest for our understanding of the role of oxidative phosphorylation in muscle function, it does not provide a very useful model of the human mitochondrial myopathies as has been previously suggested (Melmed *et al.*, 1975; Saghal *et al.*, 1979).

E. B. and D. J. H. acknowledge with gratitude fellowships and running costs provided by the Muscular Dystrophy Group and the Brain Research Trust respectively. E. A. S. was supported by a Postdoctoral Fellowship from NSERC (Canada). Some of the equipment used was provided by funds from the Mason Medical Foundation. We thank Dr. G. K. Radda for the use of n.m.r. facilities. Excellent technical assistance was given by Miss M. Ellison, Miss S. D. Nursey and Miss E. A. Wilkie.

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